PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Richard A. Dixon, et al.	Group Art Unit: 1638
Serial No.: 09/936,190	Examiner: Russell Kallis
Filed: December 9, 2001	Atty. Dkt. No.: NBLE:026US
For: GENETIC MANIPULATION OF ISOFLAVONOIDS	

DECLARATION OF RICHARD A. DIXON UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

I, RICHARD A. DIXON HEREBY DECLARE AS FOLLOWS:

1. I am a citizen of the United States and the United Kingdom and currently reside at 206 Woods Lane, Ardmore, OK 73401.

2. I have been employed by the Samuel Roberts Noble Foundation since January of 1988, currently with the position of Director of Plant Biology Division. I hold a D.Phil. in Botany from The University of Oxford. I have been conducting research in the areas of plant molecular biology and agricultural biotechnology since 1975.

3. I understand that the Patent and Trademark Office Examiner in charge of assessing the patentability of the referenced patent application has rejected the claims as not being supported by adequate information in the specification to show that introduction of a gene encoding an enzyme catalyzing the aryl migration of a flavanone would result in the production of genistein in plants that normally produce isoflavonoids.

4. I am therefore presenting the information below to show that the introduction of a gene encoding an enzyme catalyzing the aryl migration of a flavanone does in fact result in the production of genistein in plants normally producing isoflavonoids.

5. *Preparation of Transgenic Alfalfa Plants*

A binary vector construct was prepared harboring the *Medicago truncatula* isoflavone synthase coding sequence ("MtIFS") (SEQ ID NO:4 of the above patent application) under the control of a CaMV 35S promoter. The encoded enzyme catalyzes the aryl migration of a flavanone leading to the production of 2-hydroxyisoflavanones from common flavanone intermediates. The 2-hydroxyisoflavanone is unstable and will readily dehydrate to yield the isoflavones daidzein or genistein.

The vector along with an "empty" vector were transformed into alfalfa Regen SY-4D by *Agrobacterium*-mediated transformation of leaf discs using standard procedures. Transgenic plants were obtained following somatic embryogenesis and eventually transferred to the greenhouse. Approximately 50 independent lines were generated for each construct.

6. *Analysis of Transgenic Plants*

Transgenic plants had no observable phenotypic differences compared to controls. DNA gel blot analysis was used to confirm transgene presence in MtIFS-expressing lines C22 and B20, vector control lines VC11 and VB2, and non-transformed alfalfa (NT). The transgenic MtIFS-expressing alfalfa plants prepared were found to accumulate conjugates of genistein, biochanin A, and pratensein in leaves. The levels of genistein were found to be approximately five fold that of the other two isoflavones. The engineered isoflavones were present as glucosides, the main form of isoflavones in soybean, in contrast to the endogenous leaf flavones which accumulate as glucuronides.

Two lines, each harboring a single copy of the introduced transgene, were found to accumulate the highest levels of genistein (~50 nmol/g FW in the leaves), levels of which could be further increased to ~100 nmol/g FW under certain environmental conditions. No genistein was detected in the leaves of control lines.

7. The result of the above studies demonstrate that expression of an enzyme catalyzing the aryl migration of a flavanone in alfalfa results in the production genistein. As alfalfa normally produces isoflavonoids, the data indicates that expression of an enzyme catalyzing the aryl migration of a flavanone would produce genistein in other isoflavonoid-producing plants.

8. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Oct 14, 2004
Date

Richard A. Dixon
Richard A. Dixon